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PATENT APPLICATION
Attorney's Docket No.: 0838.1004-000 (MGH-1526)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Behnaz Parhami-Seren, Michael N. Margolies, and Garner T. Haupert, Jr.		
Application No.:	09/412,268	Group:	1648
Filed:	October 5, 1999	Examiner:	S. Ungar
Confirmation No.:	9455		
For:	Ouabain-Specific Monoclonal Antibodies		

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Assistant Commissioner for Patents, P.O. Box 2327, Arlington, VA 22202

on 10-9-02 Jenine Crump
Date Signature

Jenine Crump
Typed or printed name of person signing certificate

DECLARATION OF GARNER T. HAUPERT, JR., M.D. UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
P.O. Box 2327
Arlington, VA 22202

Sir:

I, Garner T. Haupert, Jr., of 512 Great Road, Littleton, Massachusetts 01460, United States of America, declare and state that:

1. I am an Associate Physician at the Massachusetts General Hospital, and an Assistant Professor of Medicine at Harvard Medical School.

2. I am a co-inventor of the invention claimed in the above-reference patent application.

3. I have studied the contents of U.S. Application No. 09/412,268 filed October 5, 1999. My study of U.S. Application No. 09/412,268 includes the claims, which have been recently amended and are directed to a monoclonal antibody or antigen binding fragment thereof having binding specificity for ouabain, wherein binding of the antibody or antigen binding fragment to ouabain is not inhibited by about 100μM (*e.g.*, 1-10, 7-1, 8E4) or 50μM (*e.g.*, 5A12) of digoxin. I have also studied the Office Action mailed from the U.S. Patent and Trademark Office on April 9, 2002.

4. This Declaration is being filed in order to address the Examiner's comments regarding Figure 6 of the subject application.

5. Figure 6 is a graph which describes data from experiments in which the inhibition of binding of mAbs 1-10 and 8E4 to immobilized antigen (ouabain-BGG coated on the wells of the microtiter plate) was studied in the presence of increasing doses of four potential inhibitors of such binding: Ouabain, digoxin, gitoxin, and digitoxin. These latter are cardiac glycosides of differing structure. In essence, if mAb 1-10 or 8E4 recognizes immunologically the cardiac glycoside and binds to it, it leaves less mAb to bind to the ouabain-BGG coated in the plate. The percent inhibition in the binding of mAb to ouabain-BGG would increase as a function of the degree of cross reactivity and the concentration (if cross reactivity exists) of the competing cardiac glycoside.

In the case of the study of potential interaction between mAbs 1-10, 8E4 and digoxin at the highest dose of digoxin shown, the data are clumped and overlapping on or just above the X (horizontal) axis. Falling on X axis indicates zero inhibition of binding of mAbs to ouabain-BGG caused by digoxin, and therefore no cross-reactivity.

When the data points are so near but not directly on the X axis (so near to zero inhibition), it is possible that the data points are within the experimental error of the

measurement method, and therefore not truly different from zero. Arriving at a determination or clarification of such a situation can be achieved using statistical analysis.

Because of the ambiguity in Figure 6 at the highest concentration of digoxin studied, we consulted the raw data in the laboratory notebook for this set of experiments, and applied a statistical analysis ("two-tailed T test") to determine the probability that the data points just above the zero line are in fact different from zero. At every concentration of digoxin tested, the absorbance value in the presence of that dose of digoxin was compared to the absorbance binding value in the absence of any inhibitor (total baseline binding), and the T test applied to determine statistically significant difference between the two values.

The generally accepted "p" value to indicate a statistically significant difference is $p \leq 0.05$. When the inhibitor was digoxin at 50 and 100 mM, the result was $p = 0.16$-0.18. This is well above the $p < 0.05$ level, indicating that the digoxin values clustered at the highest dose point in Figure 6 are in fact not different from zero inhibition.

Thus, after statistical validation, the results of the inhibition ELISA experiments represented in Figure 6 confirm the absence of digoxin cross-reactivity for mAb 1-10 found by another, more sensitive measure of Ab specificity, fluorescence quenching (Figure 5). This absence of cross reactivity was also documented by a third method, equilibrium saturation binding (see Parhami-Seren et al., J. Immunol. 1999, 163:4360-4366 (Reference AR in LTO 1449), which is our subsequent publication of the data in the referenced application).

6. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true. Moreover, these statements were made with the knowledge that willful, false statements and the like made by me are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

Garner T. Haupert, Jr., M.D.

October 9, 2002

Date

ATCC

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BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Massachusetts General Hospital
Attn: Behnaz Parhami-Seren
Building 149, 13th Street
Charlestown, MA 02129-2000

Deposited on Behalf of: The General Hospital Corporation

Identification Reference by Depositor:	**Patent Deposit Designation**
A/J mice spleen B cell hybridoma 1-10 α oua mAb	PTA-814
A/J mice spleen B cell hybridoma 8E4 α oua mAb	PTA-815

The deposits were accompanied by: __ a scientific description _ a proposed taxonomic description indicated above.
The deposits were received October 1, 1999 by this International Depository Authority and have been accepted.

AT YOUR REQUEST: X We will inform you of requests for the strains for 30 years.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested October 19, 1999. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

Barbara M. Hafley

Barbara M. Hafley, Administrator, Patent Depository

Date: October 21, 1999

cc: N. Scott Pierce, Esq. (Ref. Docket MGH-1526)